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U.S. PATENT AND TRADEMARK OFFICE
PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. §1.53(b)(2)

Atty. Docket: EIS-SCHWARTZ33

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[ ] Additional inventors are being named on separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

VACCINE COMPRISING COPOLYMER AND METHOD FOR TREATMENT OF PSYCHIATRIC DISORDERS

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ENCLOSED APPLICATION PARTS (check all that apply)

[X] Specification	Number of Pages	45	[X] Applicant claims small entity status. See 37 C.F.R. §1.27
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[X] Credit Card Payment Form PTO-2038 in the amount of $120.00 is enclosed, which covers the following fees:

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The invention was made by an agency of the United Stated Government or under a contract with an agency of the United States Government.

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Respectfully submitted,

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YEDA-053 US PROV

VACCINE COMPRISING COPOLYMER 1 AND METHOD FOR TREATMENT OF PSYCHIATRIC DISORDERS

Inventors: Michal EISENBACH-SCHWARTZ and Jonathan KIPNIS

FIELD OF THE INVENTION

The present invention relates to compositions, e.g. vaccines, comprising Copolymer 1, a Copolymer 1-related peptide, or a Copolymer 1-related polypeptide, and methods for treatment of psychiatric disorders.

BACKGROUND OF THE INVENTION

Mental disorders are now known to be characterized not only by behavioral abnormalities but also by somatic manifestations. In a number of psychiatric disorders a neurodegenerative component has been identified. In schizophrenia, for example, there is loss of hippocampal volume and death of hippocampal neurons [1,2], as well as anatomical and molecular abnormalities of excitatory neurons in the dorso-lateral prefrontal cortex[3,4]. The etiology and pathogenesis of schizophrenia are still obscure, although there is general agreement that genetic predisposition is a significant factor[5,6]. Symptoms of schizophrenia can be classified as positive, negative, and cognitive, using standard rating scales such as the Positive and Negative Symptom Scale (PANSS) [7]. Positive symptoms include hallucinations, agitation and paranoia; negative symptoms reflect the loss of interpersonal drive and normal interest in the environment; and cognitive symptoms include conceptual disorganization and disorientation. Whereas the positive symptoms usually respond well to dopamine-receptor antagonists (which however have significant and devastating side effects such as induction of Parkinson's disease), the negative

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symptoms and cognitive deficits typically persist, resulting in chronic morbidity and poor long-term outcome [8].

Until quite recently, the body's principal adaptive responses to stressful stimuli were attributed to the hypothalamic–pituitary–adrenocortical axis [9]. An association between mental disorders or acute psychological stress and the immune system has generally been considered to be unlikely or of little significance, despite a growing body of evidence suggesting that such an association not only exists, but might be a potentially important consideration in the design of therapy [10]. Studies by our group have indicated, for example, that the adaptive immune response mediated primarily by CD4-positive T cells plays a key role in protection against neurodegenerative conditions in the central nervous system (CNS) [11-13]. T cells specific to self-antigens residing in the site of CNS damage were shown to be capable of arresting or slowing down the degenerative process by producing neurotrophic factors such as brain-derived neurotrophic factor (BDNF) [14,15] and by controlling the activity of resident microglia in a way that enables them to fight off mediators of toxicity, such as glutamate, without incurring the risk of oxidative stress or harmful inflammation [16]. Boosting of the adaptive immune response by vaccination with an antigen that acts as a weak agonist of CNS-related self-proteins increases protection against a variety of toxic mediators of neurodegeneration [17]. One such antigen is the synthetic copolymer glatiramer acetate, known as Copolymer 1 or Cop-1, a random non-pathogenic synthetic copolymer composed of the four amino acids, L-tyrosine, L-glutamate, L-lysine and L-alanine, approved in several countries for the treatment of multiple sclerosis [18] under the trademark Copaxone® (a trademark of Teva Pharmaceutical Industries Ltd., Petach Tikva, Israel).

Vaccination with Cop 1 or with Cop 1-activated T cells have been shown by the present inventors to boost the protective autoimmunity, after traumatic CNS insult, thereby reducing further injury-induced damage, and can further protect CNS cells from glutamate toxicity. Reference is made to our previous United States Patent Application Serial Nos. 09/756,301 and 09/765,644, both dated 22 January,

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2001, herein incorporated by reference in their entirety as if fully disclosed herein, corresponding to WO 01/93893, which disclose that Cop 1, Cop 1-related peptides and polypeptides and T cells activated therewith protect CNS cells from glutamate toxicity (USSN 09/756,301) and prevent or inhibit neuronal degeneration or promote nerve regeneration in the CNS or PNS (USSN 09/765,644; WO 01/52878).

Prof. Schwartz and colleagues have shown that Cop 1 acts as a low-affinity antigen that activates a wide range of self-reacting T cells, resulting in neuroprotective autoimmunity that is effective against both CNS white matter and grey matter degeneration[30]. The neuroprotective effect of Cop 1 vaccination was demonstrated by the inventors in animal models of acute and chronic neurological disorders such as optic nerve injury[14], head trauma (J. Kipnis et al., 2003, *J. Neurotrauma* 20(6):559-69), glaucoma[19], amyotrophic lateral sclerosis[53] and in the applicant's patent applications WO 01/52878, WO 01/93893 and WO 03/047500.

The neuroprotective benefit conferred on degenerating neurons by Cop-1-reactive T cells apparently includes production of neurotrophins and modulation of the immune (microglial) response at the site of neurodegeneration [14,19]. Here we report that vaccination with Cop-1 can lessen behavioral abnormalities and improve cognitive function in mice suffering from glutamate or dopamine imbalance induced by MK-801 or amphetamine (AMPH).

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SUMMARY OF THE INVENTION

In one aspect, the present invention relates to a method for treatment of a individual suffering from a psychiatric disorder which comprises administering to said individual in need of such a treatment an effective amount of an agent selected

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from the group consisting of Copolymer 1, a Copolymer 1-related peptide, and a Copolymer 1-related polypeptide.

In another aspect, the present invention relates to a pharmaceitical composition, preferably a vaccine, for treatment of psychiatric disorders which comprises a pharmaceutically acceptable carrier and an agent selected from the group consisting of Copolymer 1, a Copolymer 1-related peptide, and a Copolymer 1-related polypeptide.

In a further aspect, the present invention relates to the use of an agent selected from the group consisting of Copolymer 1, a Copolymer 1-related peptide, and a Copolymer 1-related polypeptide for the preparation of a pharmaceutical composition, preferably a vaccine, for treatment of psychiatric disorders.

In still another aspect, the present invention provides an article of manufacture comprising packaging material and a pharmaceutical composition contained within the packaging material, said pharmaceutical composition comprising an agent selected from the group consisting of Copolymer 1, a Copolymer 1-related peptide, and a Copolymer 1-related polypeptide; and said packaging material includes a label that indicates that said agent is therapeutically effective for treating a psychiatric disorder.

The psychiatric disorders that may be treated according to the invention include: (i) anxiety disorders, that include phobic disorders, obsessive-compulsive disorder, post-traumatic stress disorder (PTSD), acute stress disorder and generalized anxiety disorder; (ii) mood disorders, that include depression, dysthymic disorder, bipolar disorders and cyclothymic disorder; (iii) schizophrenia and related disorders such as brief psychotic disorder, schizophreniform disorder, schizoaffective disorder and delusional disorder; (iv) drug use and dependence such as alcoholism, opiate dependence, cocaine dependence, amphetamine dependence, hallucinogen dependence, and phencyclidine use; and (v) memory loss disorders such as amnesia or memory loss associated with Alzheimer's type dementia or with non-Alzheimer's type dementia, e.g. multi-infarct dementia or memory loss

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associated with Parkinson's disease, Huntington's disease, Creutzfeld-Jakob disease, head trauma, HIV infection, hypo-thyroidism and vitamin B12 deficiency.

In preferred embodiments, the psychiatric disorder is schizophrenia, an anxiety disorder such as stress or post-traumatic stress disorder, or a mood disorder such as depression or a bipolar disorder.

In the most preferred embodiment, the individual suffering from a psychiatric disorder is immunized with Copolymer 1.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows restoration of MK-801-impaired prepulse inhibition of the acoustic startle response in mice by Cop-1 immunization. C57Bl/6j male mice were immunized with Cop-1/CFA or with PBS/CFA (sham-immunized mice). A week later these mice were injected with MK-801 (0.1 mg/kg, i.p.). The sham-immunized mice (control group) injected with MK-801 showed a significantly disrupted PPI of the acoustic startle response. In mice immunized with Cop-1/CFA, PPI was monotonically increased as a function of the prepulse intensity ($F(1,9) = 14.05$, $P < 0.005$).

Fig. 2 shows restoration of amphetamine-impaired prepulse inhibition of the acoustic startle response in mice by Cop-1 immunization. C57Bl/6j male mice were immunized as in Fig. 1. A week later these mice received a single injection of amphetamine (2.5 mg/kg i.p.). The sham-immunized mice (control group) injected with amphetamine showed a significantly disrupted PPI of the acoustic startle response. In mice immunized with Cop-1/CFA PPI was monotonically increased as a function of the prepulse intensity ($F(1,10) = 8.6$, $P < 0.015$).

Figs. 3a-3f shows performance of a spatial memory task in the Morris water maze (MWM) after injection of psychotomimetic drugs (MK-801 and amphetamine), with and without Cop-1 immunization. Injection of MK-801 (0.1 mg/kg i.p.) (Figs. 3a–c) or of amphetamine (2.5 mg/kg i.p.) (Figs. 3d–f) significantly impaired task acquisition in the MWM (increased escape latency) in

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PBS-treated mice, but not in Cop-1-treated mice. During the acquisition (Figs. 3a, 3d) and the reversal phases (Figs. 3c, 3f) of the MWM, the PBS-treated mice took significantly longer than the Cop-1 treated mice to acquire the spatial navigation task (a–c: 3-way ANOVA, repeated measures: Groups: df(1,9), $F = 56.6$, $P < 0.0001$; Trials: df(3,27), $F = 54.0$, $P < 0.00001$; Days: df(3,27), $F = 15.6$, $P < 0.00001$ for the acquisition phase and Groups: df(1,9), $F = 42.7$, $P < 0.0001$; Trials: df(3,27), $F = 24.4$, $P < 0.00001$; Days: df(1,9), $F = 7.9$, $P < 0.02$ for the reversal phase (d–f: 3-way ANOVA, repeated measures: Groups: df(1,10), $F = 9.8$, $P < 0.01$; Trials: df(3,30), $F = 29.9$, $P < 0.00001$; Days: df(1,30), $F = 21.3$, $P < 0.00001$ for the acquisition phase and Groups: df(1,10), $F = 53.7$, $P < 0.00003$; Trials: df(3,30), $F = 16.1$, $P < 0.00002$; Days: df(1,10), $F = 5.0$, $P < 0.05$ for the reversal phase).

Fig. 4 shows tracking of Cop-1-immunized and control mice in the Morris water maze after injection of MK-801. The swimming strategies of Cop-1-treated mice and PBS-treated controls differed: Cop-1-treated mice learned to swim away from the wall to search for the platform in the inner 50% of the pool and to use the platform as a refuge when they found it. Representative tracks are shown of MK-801-injected, Cop-1-immunized mice and MK-801-injected sham-immunized control mice when first tested in the MWM.

Fig. 5 shows enzyme-linked immunosorbent assay (ELISA) of brain-derived neurotrophic factor (BDNF) secreted by Cop-1-reactive T cells stimulated with specific or non-specific antigens. Cop-1-reactive T cells obtained from immunized Lewis rats were cultured for 48 h with their specific antigen in stimulation medium. T-cell supernatants were collected and subjected to sandwich ELISA. The histogram shows the concentration of secreted BDNF in each sample. Secretion of BDNF by Cop-1-reactive T cells was significantly increased after stimulation of the T cells with a specific antigen (Cop-1) or with a Cop-1-cross-reactive antigen (myelin basic protein), compared to non-stimulated T cells. Values are mean ratios ± SE (from three independent experiments) of the amounts of BDNF secreted by anti-Cop-1 T cells in response to stimulation by various antigens.

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Figs. 6a-6f show strain dependence and T-cell dependence on the ability to withstand psychological stress. (Figs. 6a, 1d) Mouse strains differ in their ability to adapt to psychological stress. A single 10-min exposure to the odor of a predator caused behavioral changes in 36.8% of male C57BL/6J mice but in only 10.5% of male BALB/c mice ($\chi 2 = 3.7$, $P<0.05$). (Figs. 6b, 6e) In the BALB/c strain, maladaptation was significantly more prevalent in SCID mice (61.9%) than in the wild type (17.2%; $\chi 2 = 10.6$, $P<0.001$). (Figs. 6c, 6f) Maladaptation was similarly more prevalent in nude mice (devoid of mature T cells only) than in the wild type (70% and 17.2%, respectively; $\chi 2$ test: $P<0.0002$), verifying that the observed differences were attributable to the absence of mature T cells.

Figs. 7a-7c show that naturally occurring CD4+CD25+ regulatory T cells suppress the ability to withstand psychological stress. (Fig. 7a) A single 10-min exposure to the odor of a predator resulted in maladaptation in 70% of nude male BALB/c mice (see **Fig. 6**). The prevalence of maladaptation was somewhat decreased (50%) in nude mice that were replenished with normal splenocytes from wild-type BALB/c mice. In nude mice that were replenished with a splenocyte population depleted of Treg, the prevalence of maladaptation (20%) was significantly lower than that of control (non-replenished) nude mice ($\chi 2=6.7$, $P<0.009$). (Fig. 7b) The startle response (mean ± SD) of nude mice replenished with splenocytes depleted of Treg was significantly weaker than that of nude mice replenished with a normal splenocyte population ($P<0.03$) or than that of control nude mice ($F(df=2,37)=9.2$, $P<0.0006$). (Fig. 7c) Nude mice replenished with splenocytes depleted of Treg spent significantly less time exploring the closed arms of the elevated plus-maze than did nude mice replenished with a normal splenocyte population ($P<0.02$) or than control nude mice ($F(df=2,37)=8.7$, $P<0.0008$).

Figs. 8a-8c are micrographs showing that the immunohistochemistry of T cells in the brain is correlated with adaptation to psychological stress. Maladapted animals from the group of nude mice replenished with a normal splenocyte population from wild-type mice and well-adapted animals from the group of nude mice replenished with splenocytes from wild-type mice depleted of Treg were killed

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and their brains were removed, perfused, embedded in paraffin, and sliced for histology. Brain slices from the hippocampal area and fimbria of the hippocampus were stained for myelinated axons with Luxol and counterstained with eosin, or stained with anti-CD3 antibodies for the presence of T cells and counterstained with hematoxylin. (Fig. 8a) Brain slices from the maladapted mice showed no staining for T cells (ii and iv). (Fig. 8b) Brain slices from the well-adapted mice showed T-cell reactivity in hippocampal areas (ii and iv) corresponding to myelin reactivity (Luxol-positive areas; i and iii). (Fig. 8c) Wild-type mice that were not exposed to stress showed, as expected, no T-cell reactivity in brain slices. The micrographs show representative results of at least 6 brain slices from each mouse, and from at least 3 mice in each group.

Fig. 9 shows the ability of mice immunized with Cop-1 to withstand psychological stress in comparison with PBS-treated mice (control), after a single 10-min exposure to the odor of a predator.

Fig. 10 shows that Cop-1 alleviates the suppressive activity mediated by Treg ($CD4^+CD25^+$). T cell proliferation was assayed by incorporation of [^{3}H]-thymidine into effector T cells co-cultured with Treg. Recorded values are from one representative experiment out of three and are expressed as means ± SD of 4 replicates.

DETAILED DESCRIPTION OF THE INVENTION

The data presented in the present application show, for the first time, that by harnessing the immune system, for example by administering a T cell-based vaccination, it is possible to partially prevent psychosis in an animal model that simulates symptoms of schizophrenia. The vaccine used here was Cop-1, a weak "universal agonist" of CNS self-antigens [30,31], capable of evoking a T-cell response which can cross-react with self-antigens. We also showed that Cop-1-reactive T cells can produce neurotrophins, in particular BDNF, upon interaction with abundant brain-specific antigens such as MBP. Production of neurotrophins,

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together with the previously demonstrated ability of homing T cells to reinforce the buffering activity of microglia at the site of CNS damage [16], might explain the observed beneficial effect of the vaccination in preventing psychosis.

Psychological trauma, like physical insults to the CNS, can cause widespread, long-term changes in neurological and neurohormonal functioning, which appear to be related to morphological changes [32,33]. There is evidence that an individual's mental or emotional state or both can directly affect immune system function [34-36]. Immune cell activity has undeservedly acquired a bad reputation in the CNS [37]. This is because, in healthy brains, the CNS is assumed to be a site of "immune privilege" [38], and in injured or diseased brains activated immune cells have often been observed in close proximity to sites of lesion [27,39,40]. Immune abnormalities have also been reported in patients with schizophrenia, and there have been numerous attempts to find a connection between schizophrenia and autoimmune disease. However, studies over the last 60 years aimed at identifying an autoimmune basis for schizophrenia have yielded no valid evidence that it exists [41].

Contrary to long-held belief, however, the effect of the immune system on the nervous system can also be beneficial. Recent studies have shown that the injured CNS can benefit from the presence of a well-controlled adaptive immunity [42,43]. T cells specifically reactive to proteins that reside in the site of the insult promote post-injury neuronal survival and restoration of function [44,45]. It was further shown that this beneficial post-injury response of autoimmune T cells to site-specific proteins is evoked spontaneously [13,46].

Our present results indeed showed that boosting of a T cell response to the self-like antigen Cop-1 significantly reduced cognitive impairment and eased psychosis in animal models with schizophrenia-associated symptoms. Both behavioral and cognitive abnormalities were observed in our mouse model within 15 minutes of administration of the psychomimetic drugs, and were counteracted by the effects of the Cop-1 vaccination given 1 week earlier. The rapidity of the Cop-1-reactive T cells in counteracting the psychotic effects of the drugs suggests that T cells had already been elicited by the immunization, and were patrolling the healthy

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brain in a non-activated state, though on alert and ready for action if needed. Alternatively, it is possible that as a result of the immunization a significant number of Cop-1-reactive T cells, as yet unactivated, were circulating in the blood, ready to home to the site of the threat when needed there. A pathological alteration in the brain level of dopamine (by injection of amphetamine) or of glutamate (by injection of MK-801) might activate these T cells, allowing rapid protection against the devastating effects of the neurotransmitter imbalance.

It seems reasonable to assume that exacerbation of both the behavioral and the cognitive manifestations of schizophrenia over time can be correlated with the neurodegeneration occurring in certain regions of the brain [47,48]. Moreover, neuronal survival in animal models injected with glutamate or glutamate antagonists such as MK-801 is T-cell dependent, as evidenced by the finding that significantly fewer neurons survive the intoxication with these agents in T cell-deficient than in normal animals [49]. Thus the same agents that experimentally induce a glutamate imbalance which causes cognitive and behavioral abnormalities can also induce neuronal degeneration. In light of these observations, coupled with the increasing recognition that neurodegeneration plays a role in schizophrenia, and on the basis of our recent data showing that autoimmune T cells are the means through which the body fights off glutamate imbalance [19], we suspect that immune-based neuroprotection might be beneficial in schizophrenia.

The mechanism underlying the protective effect of T cells against psychosis needs to be verified in further studies. Autoimmune T cells can produce several neurotrophic factors, among them BDNF [28]. T cells were also shown to shape a particular microglial and astrocytic phenotype, activated to produce neurotrophic factors, present specific antigens to their relevant T cells, and take up and dispose of excessive extracellular glutamate [16]. Easing of the psychotic effects of high concentrations of certain neurotransmitters might be a result of prevention of the neurotrasmitters from acting on neurons, or of their rapid clearance by activated microglia, or of a direct effect of T cells on the neurons, increasing their ability to withstand the neurotransmitter onslaught. The mechanisms of MK-801-mediated

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and amphetamine-mediated psychoses might be different [50]. T cells might enhance reverse uptake of the neurotransmitter glycine, thereby increasing NMDA-receptor activity [51]. Another possible indication for MK-801 therapy might be a compensatory excess of glutamate uptake by T cell-overactivated microglia or astrocytes or both, requiring therapeutic protection against overactivation of AMPA and KA glutamate receptors. These and other possible immune-related scenarios, as well as ways of preventing amphetamine-mediated psychosis, should be further investigated.

The rapidity of the T-cell response to neurotransmitter imbalance also testifies to the importance of adaptive immunity in daily maintenance of the brain. We suggest that T cells patrolling the healthy CNS represent local adaptive immunity, just as the microglia, also acting as a defense force on standby, represent local innate immunity in the CNS. Both of these cell types are probably able to sense any deviation from the normal, and to maintain homeostasis in the brain by compensating for small fluctuations [16,52]. They are not efficient enough, however, to cope with the severe abnormalities caused by injury or disease. According to this view, it can be assumed that the primary function of the autoimmune T cells in schizophrenic patients is to balance the chemical abnormalities in the CNS. However it is likely that the spontaneous response of these T cells is not strong enough, or not of the required phenotype, or not of suitable antigenic specificity to do the job. In our mouse model, boosting of this response by vaccination with a self-like antigen that directed it towards a protective phenotype and brain-specific antigens, was probably responsible for the anti-psychotic effect.

Cop-1, a drug approved by the Food and Drug Administration for use in patients with multiple sclerosis, has been shown experimentally to exert a significantly beneficial effect on neuronal survival after CNS injury and in several animal models of neurodegenerative disorders [53]. It is worth noting that since Cop-1 provides effective therapy for an autoimmune disease (MS), we can assume that it is capable of taking care of the aberrant immunity in schizophrenia regardless of whether this illness is an autoimmune disease, a syndrome of general immune

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dysfunction, or—in line with our concept, based on the present findings—a disorder of malfunctioning autoimmunity. The observed effect of Cop-1 on the alleviation of psychosis induced by psychomimetic drugs suggests that it is currently the candidate of choice for the development of a new generation of anti-psychotic drugs. Further studies are needed in order to determine the optimal vaccination regimen in patients suffering from a chronic psychiatric illness. In patients presenting for the first time with psychosis, a single therapeutic vaccination might be of substantial and long-lasting benefit.

Glutamatergic imbalance is a feature common to neurodegenerative and mental disorders. After injury to the central nervous system (CNS), T cells directed to CNS-related self-antigens participate in CNS maintenance, which includes protection against glutamate toxicity. This neuroprotective effect can be boosted by agonists of self-antigens, such as Cop-1. According to the present application, it is shown that a similar T cell-dependent mechanism is protective against neurotransmitter imbalance in the brain, leading to behavioral and cognitive malfunction. Vaccination with Cop-1 protected mice from psychotic behavior and cognitive impairment induced by MK-801 or amphetamine (simulating symptoms of schizophrenia). Cop-1-reactive T cells, upon encountering relevant self-antigens, produced brain-derived neurotrophic factor (BDNF), a neurotrophin known to be protective in schizophrenic brain, to be linked to bipolar disorder development and connected with nerve activity, memory function and mood. Our findings provide a new insight into the mind–body connection and may serve the basis for the development of a new generation of anti-psychotic treatment consisting of therapeutic vaccination.

In one preferred embodiment of the present invention, there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and Copolymer 1, most preferably in the form of its acetate salt known under the generic name glatiramer acetate. In a most preferred embodiment, the composition of the invention comprises Copaxone®, that is used for daily administration for treatment of multiple sclerosis, only that for the purpose of the present invention the

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composition is administered in a different dosage and according to a different regimen that confers neuroprotection, referred herein as neuroprotective vaccination. However, if desired, the vaccine may contain Copolymer 1 emulsified in an adjuvant suitable for human clinical use such as, for example, aluminum hydroxide, aluminum hydroxide gel, and aluminum hydroxyphosphate, or any other adjuvant that may be found suitable for human vaccination.

As used herein, the terms "Cop-1" and "Copolymer 1" are used interchangeably. For the purpose of the present invention, "Copolymer 1-related peptide or Copolymer 1-related polypeptide" is intended to include any peptide or polypeptide, including a random copolymer that cross-reacts functionally with myelin basic protein (MBP) and is able to compete with MBP on the MHC class II in the antigen presentation.

The composition or vaccine of the invention may comprise as active agent a random copolymer comprising a suitable quantity of a positively charged amino acid such as lysine or arginine, in combination with a negatively charged amino acid (preferably in a lesser quantity) such as glutamic acid or aspartic acid, optionally in combination with a non-charged neutral amino acid such as alanine or glycine, serving as a filler, and optionally with an amino acid adapted to confer on the copolymer immunogenic properties, such as an aromatic amino acid like tyrosine or tryptophan. Such compositions may include any of those copolymers disclosed in WO 00/05250, the entire contents of which being hereby incorporated herein by reference.

More specifically, the composition for use in the present invention comprises at least one copolymer selected from the group consisting of random copolymers comprising one amino acid selected from each of at least three of the following groups: (a) lysine and arginine; (b) glutamic acid and aspartic acid; (c) alanine and glycine; and (d) tyrosine and tryptophan.

The copolymers for use in the present invention can be composed of L- or D-amino acids or mixtures thereof. As is known by those of skill in the art, L-amino acids occur in most natural proteins. However, D-amino acids are commercially

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available and can be substituted for some or all of the amino acids used to make the terpolymers and other copolymers used in the present invention. The present invention contemplates the use of copolymers containing both D- and L-amino acids, as well as copolymers consisting essentially of either L- or D-amino acids.

In one embodiment of the invention, the copolymer contains four different amino acids, each from a different one of the groups (a) to (d). A preferred copolymer according to this embodiment comprises in combination alanine, glutamic acid, lysine, and tyrosine, of net overall positive electrical charge and of a molecular weight of about 2,000 - 40,000 Da, preferably of about 2,000 -13,000 Da, and is most preferably Copolymer 1 of average molecular weight of about 4,700 - 13,000 Da, but also higher molecular weight forms of Copolymer 1 are encompassed by the present invention. Preferred molecular weight ranges and processes for making a preferred form of Cop 1 are described in U.S. Patent No. 5,800,808, the entire contents of which being hereby incorporated in the entirety. It is clear that this is given by way of example only, and that the vaccine can be varied both with respect to the constituents and relative proportions of the constituents if the above general criteria are adhered to. Thus, the copolymer may be a polypeptide from about 15 to about 100, preferably from about 40 to about 80, amino acids in length, and is preferably the copolymer having the generic name glatiramer acetate.

In another embodiment, the copolymer contains three different amino acids each from a different one of three groups of the groups (a) to (d). These copolymers are herein referred to as terpolymers.

In one embodiment, the terpolymers for use in the present invention contain tyrosine, alanine, and lysine, hereinafter designated YAK. The average molar fraction of the amino acids in these terpolymers can vary. For example, tyrosine can be present in a mole fraction of about 0.005-0.250; alanine can be present in a mole fraction of about 0.3 - 0.6; and lysine can be present in a mole fraction of about 0.1-0.5. The average molecular weight is between 2,000 - 40,000 Da, and preferably between about 3,000 - 35,000 Da. In a more preferred embodiment, the average

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molecular weight is about 5,000 - 25,000 Da. It is possible to substitute arginine for lysine, glycine for alanine, and/or tryptophan for tyrosine.

In another embodiment, the terpolymers for use in the present invention contain tyrosine, glutamic acid, and lysine, hereinafter designated YEK. The average molar fraction of the amino acids in these terpolymers can vary: glutamic acid can be present in a mole fraction of about 0.005 - 0.300, tyrosine can be present in a mole fraction of about 0.005-0.250, and lysine can be present in a mole fraction of about 0.3-0.7. The average molecular weight is between 2,000 - 40,000 Da, and preferably between about 3,000 - 35,000 Da. In a more preferred embodiment, the average molecular weight is about 5,000 - 25,000 Da. It is possible to substitute aspartic acid for glutamic acid, arginine for lysine, and/or tryptophan for tyrosine.

In another embodiment the terpolymers for use in the present invention contain lysine, glutamic acid, and alanine, hereinafter designated KEA. The average molar fraction of the amino acids in these polypeptides can also vary. For example, glutamic acid can be present in a mole fraction of about 0.005 - 0.300, alanine can be present in a mole fraction of about 0.005 - 0.600, lysine can be present in a mole fraction of about 0.2 - 0.7. The average molecular weight is between 2,000 - 40,000 Da, and preferably between about 3,000 - 35,000 Da. In a more preferred embodiment, the average molecular weight is about 5,000 - 25,000 Da. It is possible to substitute aspartic acid for glutamic acid, glycine for alanine, and/or arginine for lysine.

In another embodiment, the terpolymers for use in the present invention contain tyrosine, glutamic acid, and alanine, hereinafter designated YEA. The average molar fraction of the amino acids in these polypeptides can vary. For example, tyrosine can be present in a mole fraction of about 0.005 - 0.250, glutamic acid can be present in a mole fraction of about 0.005 - 0.300, and alanine can be present in a mole fraction of about 0.005 - 0.800. The average molecular weight is between 2,000 - 40,000 Da, and preferably between about 3,000 - 35,000 Da. In a more preferred embodiment, the average molecular weight is about 5,000 - 25,000

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Da. It is possible to substitute tryptophan for tyrosine, aspartic acid for glutamic acid, and/or glycine for alanine.

In a more preferred embodiment, the mole fraction of amino acids of the terpolymers is about what is preferred for Copolymer 1. The mole fraction of amino acids in Copolymer 1 is glutamic acid about 0.14, alanine about 0.43, tyrosine about 0.10, and lysine about 0.34. The most preferred average molecular weight for Copolymer 1 is between about 5,000 - 9,000 Da. The activity of Copolymer 1 for the vaccine disclosed herein is expected to remain if one or more of the following substitutions is made: aspartic acid for glutamic acid, glycine for alanine, arginine for lysine, and tryptophan for tyrosine.

The molar ratios of the monomers of the more preferred terpolymer of glutamic acid, alanine, and tyrosine, or YEA, is about 0.21 to about 0.65 to about 0.14.

The molar ratios of the monomers of the more preferred terpolymer of glutamic acid, alanine and lysine, or KEA, is about 0.15 to about 0.48 to about 0.36. The molar ratios of the monomers of the more preferred terpolymer of glutamic acid, tyrosine, and lysine, or YEK, is about 0.26 to about 0.16 to about 0.58. The molar ratios of the monomers of the more preferred terpolymer of tyrosine, alanine and lysine, or YAK, is about 0.10 to about 0.54 to about 0.35.

The terpolymers can be made by any procedure available to one of skill in the art. For example, the terpolymers can be made under condensation conditions using the desired molar ratio of amino acids in solution, or by solid phase synthetic procedures. Condensation conditions include the proper temperature, pH, and solvent conditions for condensing the carboxyl group of one amino acid with the amino group of another amino acid to form a peptide bond. Condensing agents, for example dicyclohexylcarbodiimide, can be used to facilitate the formation of the peptide bond. Blocking groups can be used to protect functional groups, such as the side chain moieties and some of the amino or carboxyl groups against undesired side reactions.

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For example, the process disclosed in U.S. Patent 3,849,650, can be used wherein the N-carboxyanhydrides of tyrosine, alanine, γ-benzyl glutamate and N ε-trifluoroacetyl-lysine are polymerized at ambient temperatures in anhydrous dioxane with diethylamine as an initiator. The γ-carboxyl group of the glutamic acid can be deblocked by hydrogen bromide in glacial acetic acid. The trifluoroacetyl groups are removed from lysine by 1 molar piperidine. One of skill in the art readily understands that the process can be adjusted to make peptides and polypeptides containing the desired amino acids, that is, three of the four amino acids in Copolymer 1, by selectively eliminating the reactions that relate to any one of glutamic acid, alanine, tyrosine, or lysine. For purposes of this application, the terms "ambient temperature" and "room temperature" mean a temperature ranging from about 20 to about 26°C.

The molecular weight of the terpolymers can be adjusted during polypeptide synthesis or after the terpolymers have been made. To adjust the molecular weight during polypeptide synthesis, the synthetic conditions or the amounts of amino acids are adjusted so that synthesis stops when the polypeptide reaches the approximate length which is desired. After synthesis, polypeptides with the desired molecular weight can be obtained by any available size selection procedure, such as chromatography of the polypeptides on a molecular weight sizing column or gel, and collection of the molecular weight ranges desired. The present polypeptides can also be partially hydrolyzed to remove high molecular weight species, for example, by acid or enzymatic hydrolysis, and then purified to remove the acid or enzymes.

In one embodiment, the terpolymers with a desired molecular weight may be prepared by a process, which includes reacting a protected polypeptide with hydrobromic acid to form a trifluoroacetyl-polypeptide having the desired molecular weight profile. The reaction is performed for a time and at a temperature which is predetermined by one or more test reactions. During the test reaction, the time and temperature are varied and the molecular weight range of a given batch of test polypeptides is determined. The test conditions which provide the optimal molecular weight range for that batch of polypeptides are used for the batch. Thus,

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a trifluoroacetyl-polypeptide having the desired molecular weight profile can be produced by a process, which includes reacting the protected polypeptide with hydrobromic acid for a time and at a temperature predetermined by test reaction. The trifluoroacetyl-polypeptide with the desired molecular weight profile is then further treated with an aqueous piperidine solution to form a low toxicity polypeptide having the desired molecular weight.

In a preferred embodiment, a test sample of protected polypeptide from a given batch is reacted with hydrobromic acid for about 10-50 hours at a temperature of about 20-28°C. The best conditions for that batch are determined by running several test reactions. For example, in one embodiment, the protected polypeptide is reacted with hydrobromic acid for about 17 hours at a temperature of about 26°C.

As binding motifs of Cop 1 to MS-associated HLA-DR molecules are known, polypeptides of fixed sequence can readily be prepared and tested for binding to the peptide binding groove of the HLA-DR molecules. Examples of such peptides are those disclosed in WO 00/05249 and WO 00/05250, the entire contents of which being hereby incorporated herein by reference. Thirty-two of the peptides specifically disclosed in said application are reproduced in Table 1, herein below. Such peptides and other similar peptides would be expected to have similar activity as Cop-1. Such peptides, and other similar peptides, are also considered to be within the definition of Cop 1-related peptides or polypeptides and their use is considered to be part of the present invention.

The definition of "Cop 1 related-polypeptide" according to the invention is meant to encompass other synthetic amino acid copolymers such as the random four-amino acid copolymers described by M. Fridkis-Hareli et al., 2002 (J Clin Invest, 109 (12): 1635-1643), as candidates for treatment of multiple sclerosis, namely copolymers (14-, 35- and 50-mers) containing the amino acids phenylalanine, glutamic acid, alanine and lysine (poly FEAK), or tyrosine, phenylalanine, alanine and lysine (poly YFAK), and any other similar copolymer to be discovered that can be considered a universal antigen similar to Cop-1.

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Table 1

SEQ ID NO.	Peptide Sequence
1	AAAYAAAAAAKAAAA
2	AEKYAAAAAAKAAAA
3	AKEYAAAAAAKAAAA
4	AKKYAAAAAAKAAAA
5	AEAYAAAAAAKAAAA
6	KEAYAAAAAAKAAAA
7	AEEYAAAAAAKAAAA
8	AAEYAAAAAAKAAAA
9	EKAYAAAAAAKAAAA
10	AAKYEAAAAAKAAAA
11	AAKYAEAAAAKAAAA
12	EAAYAAAAAAKAAAA
13	EKKYAAAAAAKAAAA
14	EAKYAAAAAAKAAAA
15	AEKYAAAAAAAAAAA
16	AKEYAAAAAAAAAAA
17	AKKYEAAAAAAAAAA
18	AKKYAEAAAAAAAAA
19	AEAYKAAAAAAAAAA
20	KEAYAAAAAAAAAAA
21	AEEYKAAAAAAAAAA
22	AAEYKAAAAAAAAAA
23	EKAYAAAAAAAAAAA
24	AAKYEAAAAAAAAAA
25	AAKYAEAAAAAAAAA
26	EKKYAAAAAAAAAAA
27	EAKYAAAAAAAAAAA
28	AEYAKAAAAAAAAAA
29	AEKAYAAAAAAAAAA
30	EKYAAAAAAAAAAAA
31	AYKAEAAAAAAAAAA
32	AKYAEAAAAAAAAAA

The invention will now be illustrated by the following non-limiting examples.

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EXAMPLES

Materials and Methods

(i) Animals. Inbred adult male C57Bl/6J mice (8–12 weeks old) and adult female Lewis rats (8–12 weeks old) were supplied by the Animal Breeding Center of The Weizmann Institute of Science. The mice were housed in a light- and temperature-controlled room and matched for age in each experiment. Animals were handled according to the regulations formulated by IACUC (Institutional Animal Care and Use Committee).

(ii) Antigens. Copaxone (Cop-1) was purchased from Teva Pharmaceuticals (Israel).

(iii) Immunization. Each mouse was injected with a total of 100 μg of Cop-1 emulsified in an equal volume of complete Freund's adjuvant (CFA) containing 5 mg/ml of Mycobacteria H37 RA (Difco). The emulsion, in a total volume of 0.1 mL, was injected into the flank 1 week before the mouse was first injected with a psychomimetic drug. Control mice were injected with an equal volume of phosphate-buffered saline (PBS) emulsified in CFA.

(iv) T-cell lines. Ten days after Cop-1 immunization, T-cell lines were generated from draining lymph node cells obtained from Lewis rats immunized with Cop-1 emulsified in CFA. The lymph nodes were surgically removed, dissociated, washed, and then activated with the antigen (10 μg/ml) in stimulation medium as previously reported (Kipnis PNAS). The T-cell lines were expanded by repeated stimulation and propagation.

(v) Enzyme-linked immunosorbent assay. T cell-reactive Cop-1 cells were grown for 1 week in a propagation medium, then washed with PBS and re-suspended in stimulation medium. The cultured T cells (0.5×10^6 cells/mL) were then incubated, in the presence of irradiated thymocytes (10^7 cells/mL), with concanavalin A (1.25 μg/mL), MBP (10 μg/ml), Cop-1 (10 μg/mL), ovalbumin (10 μg/mL), or no antigen, in stimulation medium. After 48 h the cells were centrifuged

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and their supernatants were collected and sampled. Concentrations of brain-derived neurotrophic factor (BDNF) in the samples were determined with a sensitive sandwich ELISA. In brief, 96-well flat-bottomed plates were coated with a chicken anti-human BDNF antibody (Promega, Madison, WI) in 0.025 M $NaHCO_3$ and 0.025 M Na_2CO_3 (pH 8.2). Recombinant human BDNF (used as a standard; Research Diagnostics, Flanders, NJ) was used in serial dilutions in blocking solution containing 3% bovine serum albumin, 0.05% polyoxyethylene-sorbitan monolaurate (Tween-20), and 1% fetal calf serum in PBS (pH 8.2). Bound BDNF was detected by incubating the plates with a mouse anti-human BDNF antibody (Research Diagnostics) and then with peroxidase-conjugated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA) in blocking solution. The plates were developed using a 3,3',5,5'-tetramethyl-benzidine liquid substrate system (Sigma-Aldrich). The reaction was stopped by adding 1M H_3PO_4, and the optical density was determined at 450 nm. Results for each experiment were calculated as the amount of secreted BDNF per 1 mL of sample, after subtraction of background levels of the irradiated thymocytes incubated with the stimulation medium.

(vi) Drug solutions. Fresh solutions of dizocilpine maleate (MK-801; Sigma-Aldrich) were prepared in physiological saline (0.9% NaCl in sterile distilled water) for each batch of mice. Physiological saline was also used as a vehicle for d-amphetamine sulfate (AMPH; Sigma). AMPH (2.5 mg/kg) or saline was injected i.p. in a total volume of 5 mL/kg body weight. Mice were injected with MK-801, AMPH, or vehicle 15 min before being subjected to behavioral tests.

(vii) Morris water maze (MWM) behavioral test. Spatial memory was assessed by performance on the Morris water maze task, a hippocampal-dependent visuo-spatial learning task. Mice were given four trials per day, for 4 consecutive days, to find the hidden platform located 1.5 cm below the water surface in a pool 1.4 m in diameter. Within the testing room only distal visuo-spatial cues were available to the mice for location of the submerged platform. The escape latency, i.e., the time required by the mouse to find and climb onto the platform, was recorded for up to 60 s. Each mouse was allowed to remain on the platform for 30 s,

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and was then moved from the maze to its home cage. If the mouse did not find the platform within 120 s, it was manually placed on the platform and returned to its home cage after 30 s. The inter-trial interval was 30 s. On day 5 the platform was removed from the pool, and each mouse was tested by a probe trial for 60 s. On days 6–7 the platform was placed at the opposite location, and the mouse was retrained in four sessions. Data were recorded using an EthoVision automated tracking system (Noldus).

(viii) Prepulse inhibition. All prepulse inhibition (PPI) test sessions consisted of startle trials (pulse-alone), prepulse trials (prepulse + pulse), and no-stimulus trials (no-stim). The pulse-alone trial consisted of a 40-ms, 120-dB pulse of broadband noise. Acoustic PPI was measured by prepulse + pulse trials consisting of a 20-ms prepulse, 100 ms delay, and then a 40-ms, 120-dB startle pulse. The onset-to-onset interval was 120 ms. Acoustic prepulse intensities were 4, 8, 13, and 16 dB above the 65-dB background noise (i.e., 69, 73, 78, and 81 dB). The 'no-stim' trial consisted of background noise only. The acoustic section of the test session began and ended with five presentations of the pulse-alone trial; in between, each acoustic or 'no-stim' trial type was presented 10 times in a pseudo-random order. The average time between trials was 15 s (range: 12–30 s). After the mice were placed in the startle chambers, a 65-dB background noise was presented for a 5-min period of acclimation, and then throughout the test session.

PPI was calculated as a percentage score for each acoustic prepulse trial type: % PPI = 100 - {[(startle response for prepulse + pulse)/(startle response for pulse-alone)] × 100}. The magnitude of the acoustic startle response was calculated as the average response to all of the pulse-alone trials, excluding the first and last blocks of five pulse-alone trials each. For brevity, the main effects of prepulse intensity (which were always significant) are not discussed here. Data from the 'no-stim' trials are not included in the Results section because the values obtained were negligible relative to values from trials containing startle stimuli.

Example 1. Vaccination with Cop-1 has an anti-psychotic effect

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Dizocilpine maleate ((+)MK-801, an antagonist of the N-methyl-D-aspartate (NMDA) receptor channel), and AMPH act as psychotomimetic agents, inducing - via neurotransmitter imbalance - psychotic symptoms in healthy individuals and exacerbating psychotic symptoms in schizophrenic patients [20]. We therefore used these two compounds in an animal model to induce psychotic behavior that simulates behavioral and cellular abnormalities associated with schizophrenia [21].

One week before administration of the psychomimetic agents, each mouse was either vaccinated with Cop-1 emulsified in complete Freund's adjuvant (CFA) or sham-immunized with phosphate-buffered saline (PBS) in CFA, and then injected intraperitoneally (i.p.) with MK-801 or AMPH. The psychomimetic drug was injected 15 min before the mouse was tested for prepulse inhibition (PPI) of acoustic startle response [22]. Vaccination with Cop-1/CFA significantly reduced the abnormal PPI induced by MK-801 (**Fig. 1**) or by AMPH (**Fig. 2**). In the Cop-1/CFA-vaccinated mice, unlike in the sham-immunized control group, PPI showed a monotonic increase with increasing prepulse intensity. Thus, PPI disruptions induced by administration of each of the psychomimetic agents were prevented in part by vaccination with Cop-1/CFA.

Example 2. Cop-1 vaccination is protective against cognitive impairment induced by psychotomimetic agents

Administration of MK-801 or AMPH also induces cognitive deficits in the mice. Numerous authors have reported an MK-801-induced learning deficit in acquisition of spatial memory [23,24] and non-spatial memory tasks [25]. We therefore examined the effect of Cop-1/CFA vaccination on the ability to prevent or reverse the cognitive deficit induced by MK-801 or by AMPH.

Administration of MK-801 or AMPH significantly impaired performance of a spatial memory task in the Morris water maze (MWM), as indicated by the significantly higher escape latency in the sham-immunized mice than in the mice vaccinated with Cop1/CFA (**Fig. 3**). With each of the psychomimetic drugs the Cop1/CFA-vaccinated mice showed superior learning ability to that of the sham-

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immunized mice, which was clearly evident during days 2–3 of the experiment. During the acquisition (**Fig. 3a, d**) and the reversal (**Fig. 3 c, f**) phases of the MWM task, the sham-immunized mice took significantly longer than the Cop-1/CFA-vaccinated mice to acquire the spatial navigation task, if they were able to acquire it at all. The Cop-1/CFA-vaccinated mice learned to swim to the hidden platform and make use of it as a refuge by climbing onto it and remaining there, as indicated by decreasing latencies in successive trials. In contrast, when the sham-immunized mice encountered the hidden platform they behaved in an abnormal and maladaptive way. Even when placed directly on the hidden platform after a trial in which they had failed to locate it, these mice quickly walked or jumped off and continued swimming in a haphazard and disorganized manner. Moreover, AMPH-injected or MK-801-injected mice that were immunized with Cop1/CFA employed more methodical swimming strategies than the controls (**Fig. 4**). Thus, all of the Cop-1/CFA-vaccinated mice learned to swim away from the wall to search for the platform in the inner part of the pool and to use the platform as a refuge when they found it. In contrast, the behavior of the sham-immunized mice showed severe disturbances, including hyperactivity, swimming over the platform, and aimless swimming in circles. Subsequent performance of the mice in the elevated-plus maze task indicated, however, that the observed differences in spatial navigation ability in the MWM task between the Cop-1 immunized and the sham-immunized mice was not due to a difference in anxiety between the two groups. Mice that were vaccinated with Cop-1 prior to MK-801 injection also showed better "communicative" behavior than the sham-immunized controls in the social behavior test (data not shown).

Example 3. Production of BDNF by Cop-1-reactive T cells upon encountering brain proteins.

Several research groups have reported that T cells reactive to Cop-1 home to sites of pathology in the CNS [14,26]. Such T cells, being able to cross-react with various CNS-related self-antigens, can produce neurotrophic factors such as BDNF,

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which is known for its ability to confer neurotrotection of injured CNS tissue [27]. BDNF was also shown to reduce symptoms of schizophrenia [28,29]. We therefore carried out an experiment *in vitro* to determine whether Cop-1-reactive T cells, on encountering CNS myelin, can produce BDNF. **Fig. 5** shows that the production of BDNF by Cop-1-reactive T cells was increased when these T cells encountered their specific antigen (Cop-1) or the CNS-related self-antigen myelin basic protein (MBP).

Protection against consequences of psychological trauma is T-cell dependent and is suppressed by naturally occurring CD4+CD25+ regulatory T cells

Protection against neurodegenerative conditions in the central nervous system (CNS) is T-cell dependent. Here we examined whether T cells also play a role in the ability of mice to withstand psychological stress (caused, for example, by predator odor) associated with behavioral changes reminiscent of posttraumatic stress disorder (PTSD). Measurement of behavioral adaptation (acoustic startle response and avoidance behavior) in mice after their exposure to predator odor revealed that maladaptation was significantly more prevalent (χ^2=10.6, P<0.001) in immune-deficient mice (62%) than in their wild-type counterparts (17%). The prevalence of maladaptation in the normal mice was reduced upon removal of naturally occurring CD4+CD25+ regulatory T cells, which normally suppress autoimmunity. The ability to cope with stress was correlated with recruitment of T cells in the brain. These findings suggest that a well-controlled T cell-dependent dialog between the brain and the immune system is needed for *mens sana in corpore sano*.

Materials and Methods

Animals. Inbred adult wild-type and *nu/nu* BALB/c and C57Bl/6J mice were supplied by the Animal Breeding Center of The Weizmann Institute of Science. All

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animals were handled according to the regulations formulated by IACUC (Institutional Animal Care and Use Committee).

Induction of psychological stress. Mice in the experimental groups were exposed for 10 minutes to thoroughly soiled cat litter (used by a cat for 2 days and sifted for faeces). Control (WT) mice were exposed for the same time to unused litter.

Study design for determination of cut-off behavioral criteria. The study was designed in two steps. The first was intended to test the zero hypothesis, i.e. that exposure to the odor of predator urine would affect all the mice in the experimental group relative to controls, but there would nevertheless be a range of behavioral effects that would serve as a basis for the definition of cut-off behavioral criteria (CBC's) for both groups. The data obtained for two groups would then provide the basis for the definition of CBC in the second step.

Procedure. Each 5-min session was recorded using an overhead video camera connected to a monitor/recorder in an adjacent observation room. Taped sessions were reviewed and behaviors rated by observers who were blinded to the group allocations. Five behavioral parameters were assessed: (1) time spent in the open arms; (2) time spent in the closed arms; (3) number of entries into open arms; (4) number of entries into closed arms; (5) total number of entries into all arms. Mice were recorded as having entered an open or closed arm only when all four paws had passed over the dividing line between open and closed arms. "Exploration activity" was defined and calculated as the number of entries into any arm of the maze (total arm entries).

Acoustic startle response (ASR). Pairs of mice were tested in startle chambers. The startle response and pre-pulse inhibition were measured using two ventilated startle chambers (SR-LAB system, San Diego Instruments, San Diego, CA). Each chamber consists of a Plexiglas cylinder resting on a platform inside a sound-attenuated, ventilated chamber. A high-frequency loudspeaker inside the chamber produces both a continuous broad-band background noise of 68 dB and different acoustic stimuli. Movement inside the tube is detected by a piezoelectric

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accelerometer located below the frame. The amplitude of the startle response of the whole body to an acoustic pulse is defined as the average of 100 accelerometer readings, 100 ms each, collected from pulse onset. These readings (signals) are digitized and stored in a computer. Sound levels within each test chamber are routinely measured using a sound-level meter (Radio Shack, San Diego Instruments) to ensure consistent presentation. An SR-LAB calibration unit is used routinely to ensure consistency of the stabilimeter sensitivity between test chambers and over time. The mice were placed inside the tube, and the startle session started with a 5-min acclimatization period, to the background noise level of 68 dB, which was maintained throughout the session, as specified above.

Antibodies and reagents. Mouse recombinant IL-2 (mrIL-2) and anti mouse ζ-CD3 (clone 145-2C11) were purchased from R&D Systems (Minneapolis, MN). Rat anti-mouse phycoerythrin (PE)-conjugated CD25 antibody (PC61) was purchased from Pharmingen (Becton-Dickinson, Franklin Lakes, NJ).

Histology and immunohistochemistry of paraffin-embedded brain sections. Paraffin-embedded brain tissues from maladapted nude mice replenished with a normal population of wild-type splenocytes, or from well-adapted nude mice replenished with wild type splenocytes depleted of CD25+ regulatory T cells, were cut into 4-μm-thick coronal sections, deparaffinized with xylene, and dehydrated with a graded series of ethanol solutions. The sections were then stained with Luxol fast blue (Sigma-Aldrich, Israel) and counterstained with Fast Red (Sigma, Israel). For immunohistochemical analyses, deparaffinized and dehydrated sections were immersed (30 min) in methanol containing 3% H_2O_2 and 1% of concentrated HCl to block endogenous peroxidase activity, treated (1 h) with phosphate-buffered saline (PBS), pH 7.4, containing 20% normal rabbit serum and 0.3% Triton X-100, and incubated overnight at room temperature with anti-CD3 antibodies (Serotec, Oxford, UK; diluted 1:50 in PBS containing 2% normal rabbit serum). The sections were washed with PBS and incubated for 30 min, first with biotinylated anti-rabbit IgG and then with avidin-biotinylated peroxidase complex (Vector Laboratories,

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Burlingame, CA). Peroxidase activity in a solution of 3,3'-diaminobenz-iodine was visualized by light microscopy.

Preparation of splenocytes. Donor splenocytes from rats (aged up to 10 weeks) were obtained by rupturing the spleen and following conventional procedures. The splenocytes were washed with hypotonic buffer (ACK) to lyse red blood cells.

Depletion of CD25+ cells. Splenocytes obtained from wild-type mice were prepared by the standard procedure and incubated with rat anti-mouse PE-conjugated CD25 antibody and then with anti-PE beads (Becton-Dickinson). The washed splenocytes were subjected to AutoMacs (Miltenyi Biotec, Gladbach, Germany) using the 'deplete sensitive' program. Recovered populations were analyzed by FACSort.

Preparation of lymphocytes. Mouse donor lymph nodes (axillary, inguinal, superficial cervical, mandibular, and mesenteric) were ruptured through mesh. The lymphocytes were washed with ACK buffer to lyse red blood cells.

Example 4. Strain dependence and T-cell dependence on the ability to withstand psychological stress

Psychological trauma, like physical CNS insult, can cause widespread long-term changes in neurological and neurohormonal functioning, and appears to be related to structural changes [32,33]. There is evidence that mental/emotional state directly affects the immune system status [34,35,36]. It was therefore of interest to examine the effect of CD4+ (adaptive immunity) T cells on the ability to withstand psychological trauma.

Previous studies have shown that exposure of rats or mice to a predator (cat) or odor of a predator (thoroughly soiled cat litter) for 10 minutes causes major stress in these animals (Adamec et al., 1999; Adamec and Shallow, 1993; Cohen *et al.*, 2003; Cohen et al., 2000). In the present application we used this stress model. We first exposed naïve adult mice of two strains (C57Bl/6J and BALB/c) to the odor of a cat, as previously described (Cohen et al., 2003). Seven days later we assessed

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their behavioral responses to two sequentially administered behavioral challenges, the elevated plus-maze and the acoustic startle response, which together provide a framework for selected cutoff behavioral criteria (CBC). By classifying the tested mice as either "maladapted" or "well adapted", we could determine the prevalence of the more severely affected animals.

The two strains (C57Bl/6J and BALB/c) differed in their overall adaptation to the imposed psychological trauma (**Figs. 6a–c**). The incidence of maladaptation in C57Bl/6J mice was 36.8%, whereas in BALB/c mice it was only 10.5% (χ^2=3.7, $P < 0.05$; **Fig. 6a**). The differences were significantly manifested in the acoustic startle response (**Fig. 6b**), but not in the time spent in the closed arms of the elevated plus-maze (**Fig. 6c**). This finding is in line with previous observations in connection with the strain-related ability of mice to withstand glutamate toxicity and optic nerve injury (Kipnis et al., 2001).

The results prompted us to investigate whether the adaptive immune system (represented by CD4+ T cells) affects the behavioral consequences of traumatic mental stress and the adaptation to such stress. We therefore examined the ability to adapt to the psychological stress in the absence of well-functioning immune system. Because of the relatively low incidence of maladaptation in the BALB/c mice (**Fig. 6a**) we used this strain to compare the response to stress in the wild type (WT) to that in mice with severe combined immune deficiency (SCID) with the same genetic background. Significantly more mice showed symptoms of maladaptation in the SCID mice than in the WT (61.9% compared to 17.2%; χ^2 =10.6, $P < 0.001$; **Fig. 6d**. The same comparison between nude mice (devoid of mature T cells only) and the WT data yielded similar results (70% compared to 17.2%; χ^2 =13.9, P<0.0002; **Fig. 6d**, verifying that the observed differences were due to the absence of mature T cells. Differences between the wild type mice and both the SCID and the nude mice were significantly manifested in the acoustic startle response (**Fig. 6 e**) as well as in the time spent in the closed arms of the elevated plus-maze (**Fig. 6f**).

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EXAMPLE 5. Naturally occurring CD4+CD25+ regulatory T cells suppress the ability to withstand psychological stress

The spontaneous ability to fight off the sequelae of a mechanical (e.g. crush) injury or a biochemical insult (e.g. from glutamate toxicity) to the CNS [46] is suppressed by naturally occurring regulatory CD4+CD25+ T cells (Treg), which comprise approximately 10% of the CD4+ T-cell population and are thought to be responsible for peripheral tolerance of autoimmune T cells. We therefore postulated that the same population of regulatory T cells also suppresses the ability to withstand psychological trauma. Comparison of nude BALB/c mice reconstituted with whole splenocytes and nude BALB/c mice replenished with splenocytes devoid of Treg showed that the incidence of maladaptation in the latter group of mice (20%) was significantly lower than in the former (50%) (χ^2=4.0, P<0.046; **(Fig. 7a)**. The differences observed between the two groups were significant both in the acute startle response **(Fig. 7b)** and in the time spent in closed arms of the maze **(Fig. 7c)**.

EXAMPLE 6. Immunohistochemistry of T cells in the brain is correlated with adaptation to psychological stress

In mice and rats suffering from neurodegenerative conditions, the beneficial effect of T cells is correlated with T cell accumulation at the site of the lesion (Moalem et al., 1999; Hauben et al., 2000; Kipnis et al., 2000; Butovsky et al., 2001). To determine whether the observed beneficial effect of the T cell response on the outcome of the stressful psychological conditions is also exerted, at least in part, by homing of T cells to the CNS, we compared the results of immunocytochemical staining for T cells in brain slices from well-adapted nude BALB/c mice that were replenished with splenocytes depleted of Treg and those from maladapted BALB/c mice injected with a normal splenocyte population. Staining of brain slices with hematoxylin and eosin revealed no structural alterations in the hippocampus or amygdala (data not shown). Luxol staining also showed no differences between maladapted **(Fig. 8a*i* and 8a*iii*)** and well-adapted mice **(Fig. 8bi and 8b*iii*)**

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compared to WT (**Fig. 8c*i***). Staining with anti-CD3 antibodies, however, revealed large numbers of T cells in these brain regions of well-adapted mice (**Fig. 8a*ii* and 8a*iv***) and hardly any in maladapted (**Fig. 8b*ii* and 8b*iv***) or normal WT mice (**Fig. 8c*ii***), suggesting that to the recruitment of T cells to the brain is correlated with the resistance to mental stress.

Example 7. Behavioral adaptation to psychological stress.

Fig. 9 shows the ability of mice immunized with Cop-1 to withstand psychological stress in comparison with PBS-treated mice (control), after a single single 10-min exposure to the odor of a predator. It can be seen that this single exposure caused behavioral changes in 40.8% of male C57Bl/6J control mice (immunized with PBS emulsified in CFA) and in only 14.8% of Cop-1 immunized mice. Maladaptation, resembling posttraumatic stress disorder (PTSD) symptoms. was more prevalent in control mice than in Cop-1 immunized mice ($P<0.05$), verifying that the observed differences were attributable to the presence of protective Cop-1 reactive T cells.

Example 8. Cop-1 alleviates the suppressive activity mediated by $CD4^+CD25^+$ regulatory T cells (Treg)

In this experiment, Treg were activated by incubation for 24 h with anti-CD3 antibodies in the presence of mrIL-2, with or without Cop-1. **Fig. 10** shows that incubation of the activated Treg for 2 h with poly Cop-1 prior to their co-culturing with Teff alleviated the suppression of Teff compared to that obtained with Treg not exposed to Cop-1. Recorded values are from one representative experiment out of three and are expressed as means ± SD of 4 replicates.

Discussion

To the best of our knowledge, this is the first demonstration that cross-talk between the brain and the adaptive immune system (T cells) affects the consequences of a single instance of psychological trauma. Complete T cell

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deficiency was found here to correlate with maladaptation to psychological stress, whereas removal of only a subpopulation of T cells, the naturally occurring suppressor T cells (Treg), improved the ability to adapt to the stress. According to our interpretation, in normal animals subjected to traumatic mental stress, the T cell-mediated response can't reach its full therapeutic potential, as it is suppressed by the presence of the naturally occurring regulatory T cells.

The precise mechanism underlying the posttraumatic sequelae of psychological trauma is not fully known, nor is it clear whether or how neural death takes place under such conditions. Recent studies strongly suggest that tissues in need of repair derive benefit from the presence of T cells. Such T cells, once locally activated by encountering their relevant antigens, control microglia behavior needed for fighting off local enemies emerging within the stressful brain [16]. The presence of T cells in brain regions after exposure of the individual to a psychological stressor might serve as an indication of regions in need of repair. We showed here that under conditions of psychological stress the recruitment of T cells to the brains of maladapted mice was poor, whereas the presence of recruited T cells was clearly observed in brain regions of the mice that were well adapted to stress.

As demonstrated in a model of acute and chronic neurodegenerative disorders [45], one way to boost a neuroprotective response to neural tissue damage is by immunization with an self-antigen that resides in the site of damage, causing its specific autoimmune T cells to home to the distressed site. Another way to boost such a neuroprotective response is by depletion of Treg, the sub-population of T cells that normally restrains the response mediated by autoimmune T cells. Depletion of Treg increases the ability of mice to reject tumors and to withstand CNS injuries [30], as it allows activation of T cells directed against self-antigens, and in particular against those associated with the intimidation such as tumors in the former case and with those resident at the site of the CNS injury in the latter. In the case of psychological stress, neither the precise location of the damaged area nor the identity of the relevant antigens is known. Nevertheless, the observed salutary effect of Treg depletion on the ability to contain adverse mental conditions, the ability to

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reject tumors, and the ability to withstand CNS injuries suggests that this T-cell subpopulation plays a key role in the cross-talk that regulates a fundamental part of the body's system of maintenance.

The behavioral outcome in mice exposed to the odor of a predator has been used as a model for posttraumatic stress disorder (PTSD). Brief, escapable exposure of mice or rats to a cat or cat odor increases defensive behaviors observed in a visible burrow system for many hours after removal of the threat. Predatory stimuli are ecologically relevant for an animal's survival and, consequently induce responses quite similar to those exerted in natural contexts.

The mechanism underlying PTSD is still a subject of controversy. One theory postulates high concentrations of glutamate in several brain regions, such as the amygdala. We were unable to detect morphological signs of neuronal degeneration in the brains of the mice 1 week after exposure to predator odor. It should be borne in mind, however, that a 1-week interval is probably too short to allow meaningful assessment of the morphological consequences of a psychological stress. It is possible that once a steady state is reached and the disease becomes chronic, some neuronal loss will occur. It is also possible, however, that the stress is for the most part manifested functionally rather than in gross histology, at least in the acute phase, and for the most part manifested—and alleviated—functionally rather than in gross morphology.

The results of this study suggest that the inherent mode of functioning of the immune system might contribute to either the susceptibility or the resistance of the individual to PTSD after a traumatic event. These findings thus strongly suggest that T cells are playing role in the dialogue between the mind and the body, and that, as the ancient Romans told us, *mens sana in corpore sano*—a healthy mind is a function of a healthy body. We would add that, in particular, it is a function of a properly operating immune system, in which the naturally occurring regulatory T cells play a pivotal role in maintaining the balance between the need for adaptive autoimmunity as a way of recruiting help to rescue tissues in distress and the need to avoid unnecessary risk of autoimmune disease. It seems that while the

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immune–mind–body connection works well under normal conditions, the outcome can be devastating if the immune system is malfunctioning or the stress is too severe to be contained by the individual's constitutive immunity.

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CLAIMS:

1. A method for treatment of a patient suffering from a psychiatric disorder which comprises administering to said individual in need of such a treatment an effective amount of an agent selected from the group consisting of Copolymer 1, a Copolymer 1-related peptide, and a Copolymer 1-related polypeptide.

2. A method according to claim 1 wherein said patient is immunized with a therapeutically effective amount of an agent selected from the group consisting of Copolymer 1, a Copolymer 1-related peptide, and a Copolymer 1-related polypeptide.

3. A method according to claim 1 or 2, wherein said agent is Copolymer 1.

4. A method according to claim 1 or 2, wherein said agent is a Copolymer 1-related peptide or a Copolymer 1-related polypeptide.

5. A method according to any one of claims 1 to 4 wherein said psychiatric disorder is selected from: (i) anxiety disorders, that include phobic disorders, obsessive-compulsive disorder, post-traumatic stress disorder (PTSD), acute stress disorder and generalized anxiety disorder; (ii) mood disorders, that include depression, dysthymic disorder, bipolar disorders and cyclothymic disorder; (iii) schizophrenia and related disorders such as brief psychotic disorder, schizophreniform disorder, schizoaffective disorder and delusional disorder; (iv) drug use and dependence such as alcoholism, opiate dependence, cocaine dependence, amphetamine dependence, hallucinogen dependence, and phencyclidine use; and (v) memory loss disorders such as amnesia or memory loss associated with Alzheimer's type dementia or with non-Alzheimer's type dementia, e.g. multi-infarct dementia or memory loss associated with Parkinson's disease, Huntington's disease, Creutzfeld-Jakob disease, head trauma, HIV infection, hypo-thyroidism and vitamin B12 deficiency.

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6. The method according to claim 5 wherein said psychiatric disorder is schizophrenia, an anxiety disorder such as stress or post-traumatic stress disorder, or a mood disorder such as depression or a bipolar disorder.

7. A pharmaceutical composition for treatment of psychiatric disorders comprising a pharmaceutically acceptable carrier and an active agent selected from the group consisting of Copolymer 1, a Copolymer 1-related peptide, and a Copolymer 1-related polypeptide.

8. A pharmaceutical composition according to claim 7, wherein said active agent is Copolymer 1.

9. A pharmaceutical composition according to claim 8, wherein said agent is a Copolymer 1-related peptide or a Copolymer 1-related polypeptide.

10. A pharmaceutical composition according to any one of claims 7 to 9 wherein said psychiatric disorder is selected from: (i) anxiety disorders, that include phobic disorders, obsessive-compulsive disorder, post-traumatic stress disorder (PTSD), acute stress disorder and generalized anxiety disorder; (ii) mood disorders, that include depression, dysthymic disorder, bipolar disorders and cyclothymic disorder; (iii) schizophrenia and related disorders such as brief psychotic disorder, schizophreniform disorder, schizoaffective disorder and delusional disorder; (iv) drug use and dependence such as alcoholism, opiate dependence, cocaine dependence, amphetamine dependence, hallucinogen dependence, and phencyclidine use; and (v) memory loss disorders such as amnesia or memory loss associated with Alzheimer's type dementia or with non-Alzheimer's type dementia, e.g. multi-infarct dementia or memory loss associated with Parkinson's disease, Huntington's disease, Creutzfeld-Jakob disease, head trauma, HIV infection, hypothyroidism and vitamin B12 deficiency.

11. A pharmaceutical composition according to claim 10 wherein said psychiatric disorder is schizophrenia, an anxiety disorder such as stress or post-

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traumatic stress disorder, or a mood disorder such as depression or a bipolar disorder.

12. A vaccine for immunization of an individual suffering from a psychiatric disorder comprising an active agent selected from the group consisting of Copolymer 1, a Copolymer 1-related peptide, and a Copolymer 1-related polypeptide.

13. A vaccine according to claim 12, wherein said active agent is Copolymer 1.

14. A vaccine according to claim 12, wherein said agent is a Copolymer 1-related peptide or a Copolymer 1-related polypeptide.

15. A vaccine according to any one of claims 12 to 14, wherein said vaccine comprises the active agent without an adjuvant.

16. A vaccine according to any one of claims 12 to 15, wherein said vaccine comprises the active agent emulsified in an adjuvant suitable for human clinical use.

17. A vaccine according to claim 16, wherein said adjuvant is selected from the group consisting of aluminum hydroxide, aluminum hydroxide gel, and aluminum hydroxyphosphate.

18. A vaccine according to claim any one of claims 12 to 17 for immunization of an individual suffering from a psychiatric disorder selected from: (i) anxiety disorders, that include phobic disorders, obsessive-compulsive disorder, post-traumatic stress disorder (PTSD), acute stress disorder and generalized anxiety disorder; (ii) mood disorders, that include depression, dysthymic disorder, bipolar disorders and cyclothymic disorder; (iii) schizophrenia and related disorders such as brief psychotic disorder, schizophreniform disorder, schizoaffective disorder and delusional disorder; (iv) drug use and dependence such as alcoholism, opiate dependence, cocaine dependence, amphetamine dependence, hallucinogen dependence, and phencyclidine use; and (v) memory loss disorders such as amnesia

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or memory loss associated with Alzheimer's type dementia or with non-Alzheimer's type dementia, e.g. multi-infarct dementia or memory loss associated with Parkinson's disease, Huntington's disease, Creutzfeld-Jakob disease, head trauma, HIV infection, hypo-thyroidism and vitamin B12 deficiency.

19. A vaccine according to claim 18 wherein said psychiatric disorder is schizophrenia, an anxiety disorder such as stress or post-traumatic stress disorder, or a mood disorder such as depression or a bipolar disorder.

20. Use of an agent selected from the group consisting of Copolymer 1, a Copolymer 1-related peptide, and a Copolymer 1-related polypeptide, for the preparation of a pharmaceutical composition for treatment of a psychiatric disorder.

21. Use of an agent selected from the group consisting of Copolymer 1, a Copolymer 1-related peptide, and a Copolymer 1-related polypeptide, for the preparation of a vaccine for immunization of an individual suffering from a psychiatric disorder.

22. Use according to claim 21 wherein said vaccine comprises the active agent without an adjuvant.

23. Use according to claim 22 wherein said vaccine comprises the active agent emulsified in an adjuvant suitable for human clinical use.

24. Use according to claim 23, wherein said adjuvant is selected from the group consisting of aluminum hydroxide, aluminum hydroxide gel, and aluminum hydroxyphosphate.

25. Use according to any one of claims 20 to 24 wherein said active agent is Copolymer 1.

26. Use according to any one of claims 20 to 24 wherein said active agent is a Copolymer 1-related peptide or a Copolymer 1-related polypeptide.

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27. Use according to any one of claims 20 to 24 wherein said psychiatric disorder is selected from: (i) anxiety disorders, that include phobic disorders, obsessive-compulsive disorder, post-traumatic stress disorder (PTSD), acute stress disorder and generalized anxiety disorder; (ii) mood disorders, that include depression, dysthymic disorder, bipolar disorders and cyclothymic disorder; (iii) schizophrenia and related disorders such as brief psychotic disorder, schizophreniform disorder, schizoaffective disorder and delusional disorder; (iv) drug use and dependence such as alcoholism, opiate dependence, cocaine dependence, amphetamine dependence, hallucinogen dependence, and phencyclidine use; and (v) memory loss disorders such as amnesia or memory loss associated with Alzheimer's type dementia or with non-Alzheimer's type dementia, e.g. multi-infarct dementia or memory loss associated with Parkinson's disease, Huntington's disease, Creutzfeld-Jakob disease, head trauma, HIV infection, hypothyroidism and vitamin B12 deficiency.

28. Use according to claim 27 wherein said psychiatric disorder is schizophrenia, an anxiety disorder such as stress or post-traumatic stress disorder, or a mood disorder such as depression or a bipolar disorder.

29. An article of manufacture comprising packaging material and a pharmaceutical composition contained within the packaging material, said pharmaceutical composition comprising an agent selected from the group consisting of Copolymer 1, a Copolymer 1-related peptide, and a Copolymer 1-related polypeptide; and said packaging material includes a label that indicates that said agent is therapeutically effective for treating a psychiatric disorder.

30. An article of manufacture comprising packaging material and a pharmaceutical composition contained within the packaging material, said pharmaceutical composition comprising Copolymer 1; and said packaging material includes a label that indicates that Copolymer 1 is therapeutically effective for treating a psychiatric disorder.

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31. The article of manufacture of claim 29 or 30 wherein said psychiatric disorder is selected from: (i) anxiety disorders, that include phobic disorders, obsessive-compulsive disorder, post-traumatic stress disorder (PTSD), acute stress disorder and generalized anxiety disorder; (ii) mood disorders, that include depression, dysthymic disorder, bipolar disorders and cyclothymic disorder; (iii) schizophrenia and related disorders such as brief psychotic disorder, schizophreniform disorder, schizoaffective disorder and delusional disorder; (iv) drug use and dependence such as alcoholism, opiate dependence, cocaine dependence, amphetamine dependence, hallucinogen dependence, and phencyclidine use; and (v) memory loss disorders such as amnesia or memory loss associated with Alzheimer's type dementia or with non-Alzheimer's type dementia, e.g. multi-infarct dementia or memory loss associated with Parkinson's disease, Huntington's disease, Creutzfeld-Jakob disease, head trauma, HIV infection, hypothyroidism and vitamin B12 deficiency.

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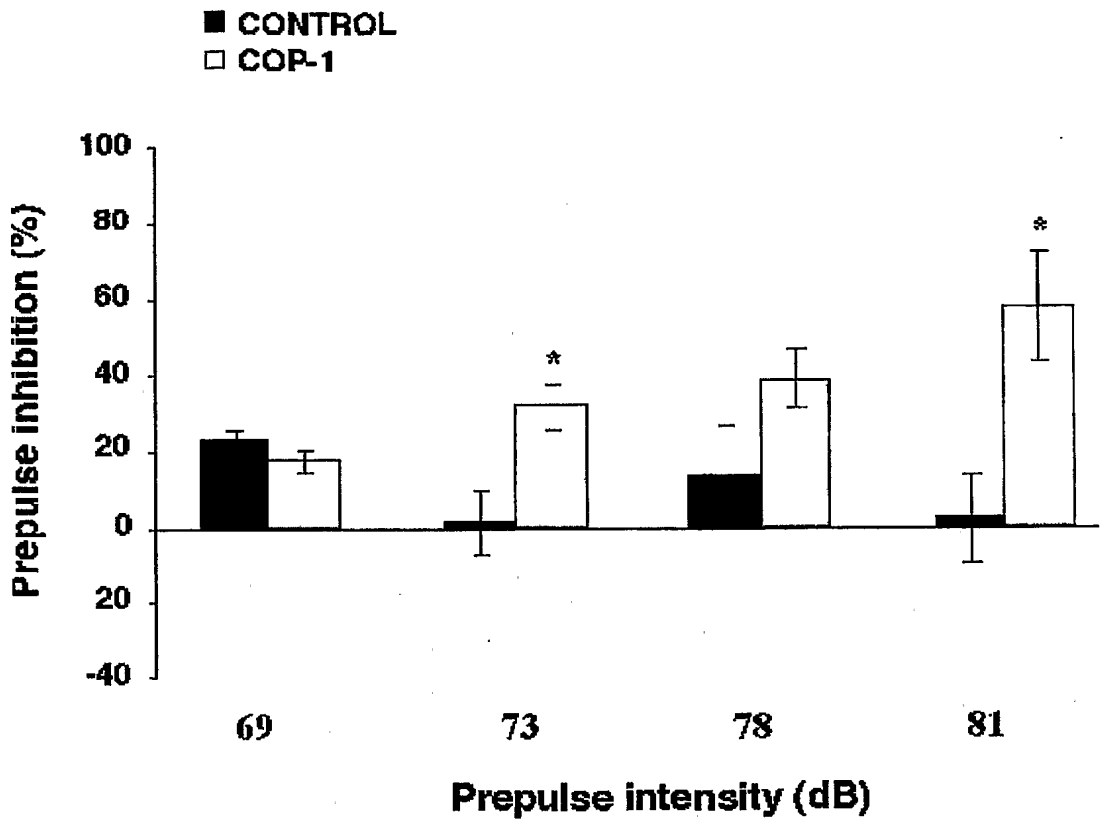

Fig. 1

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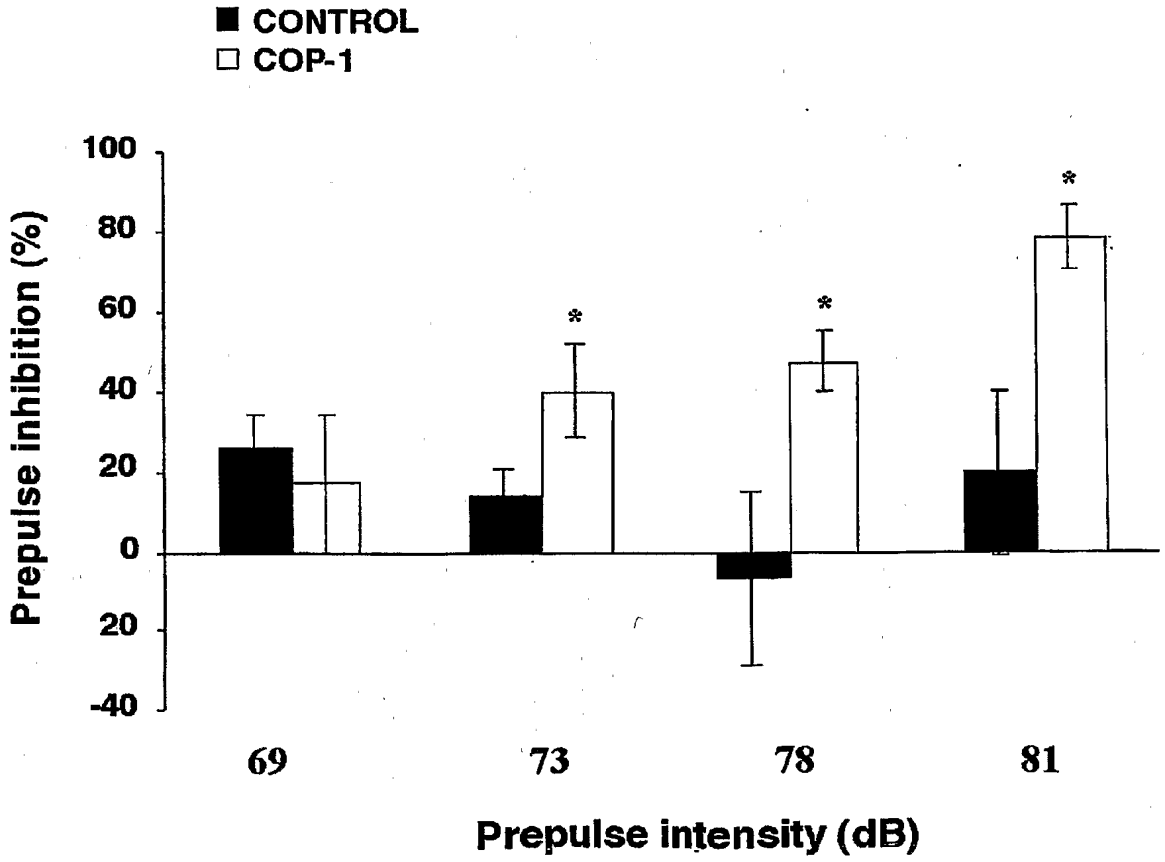

Fig. 2

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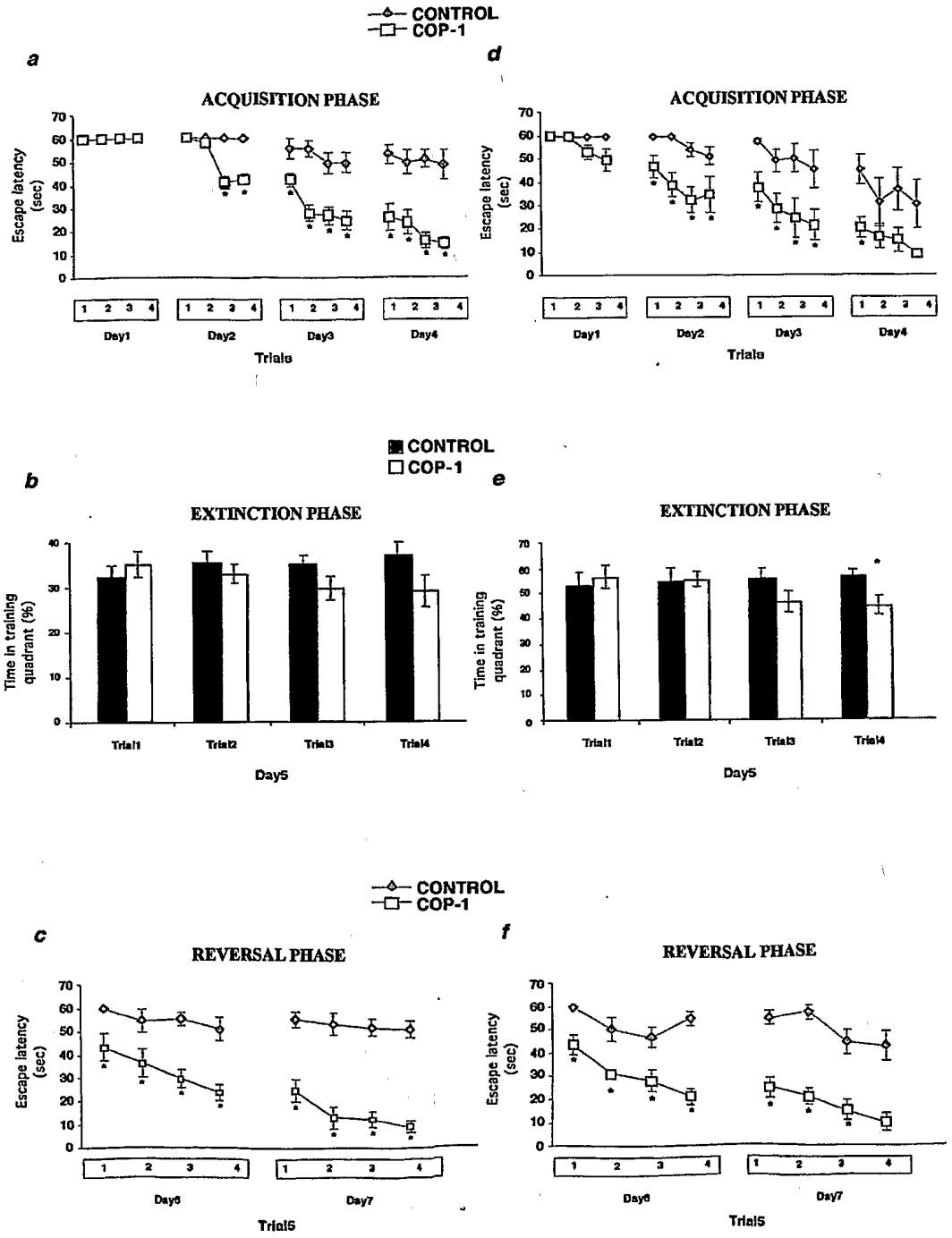

Fig. 3

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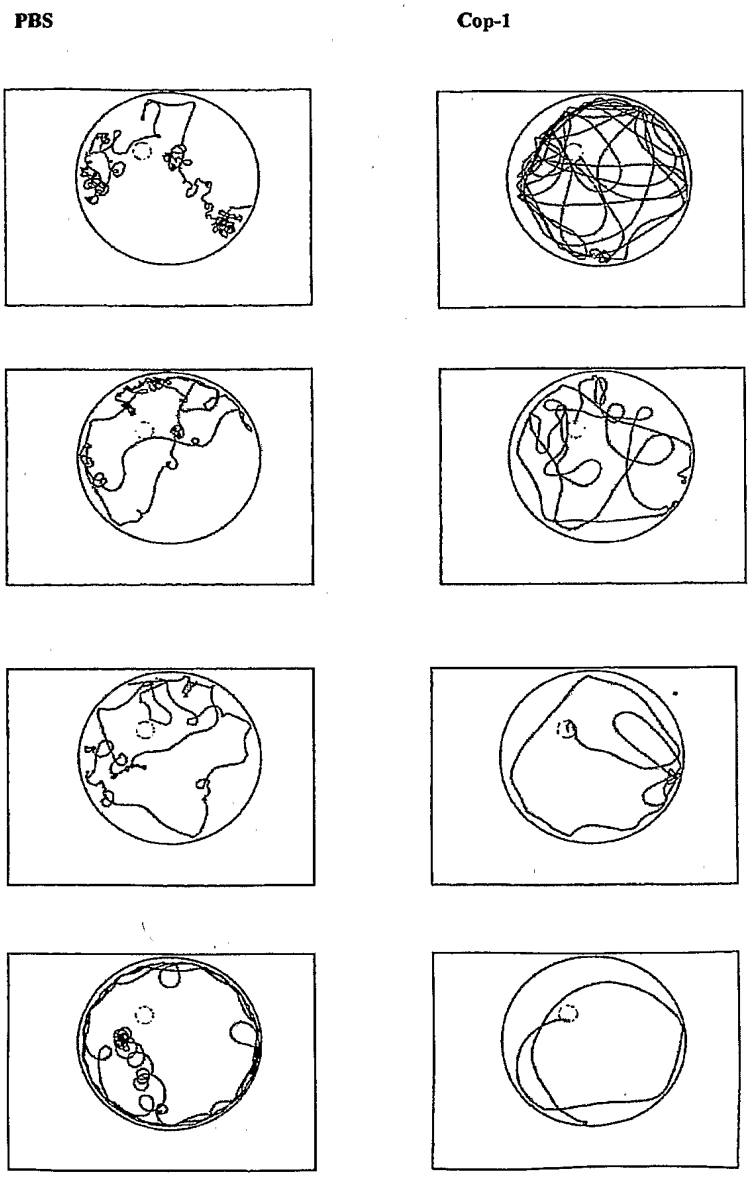

Fig. 4

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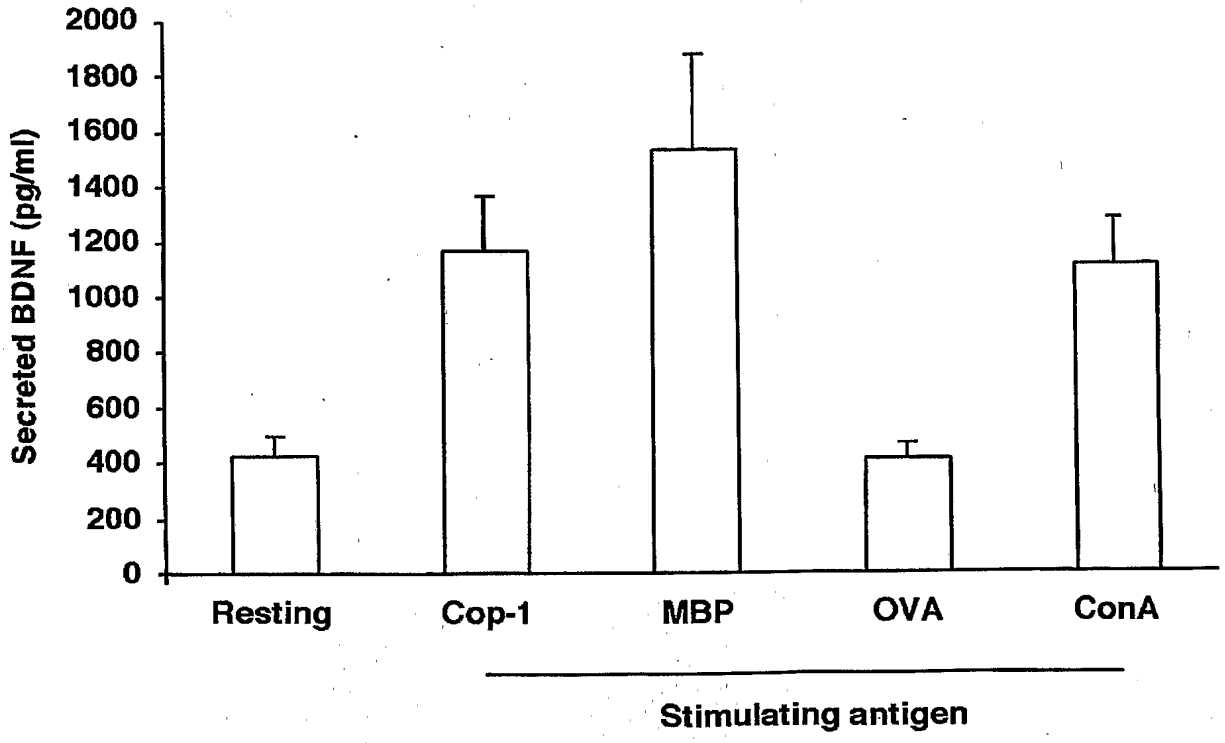

Fig. 5

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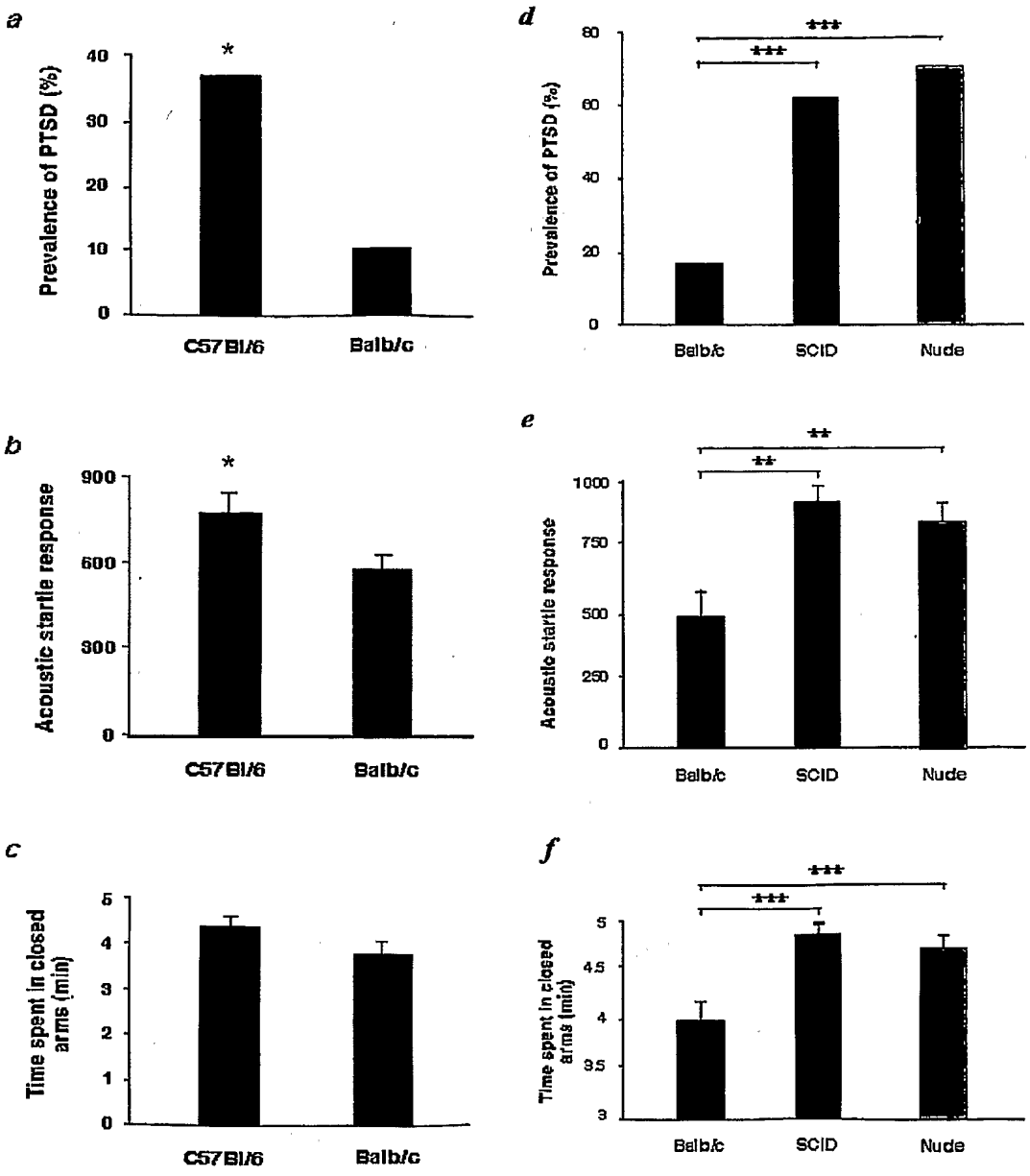

Fig. 6

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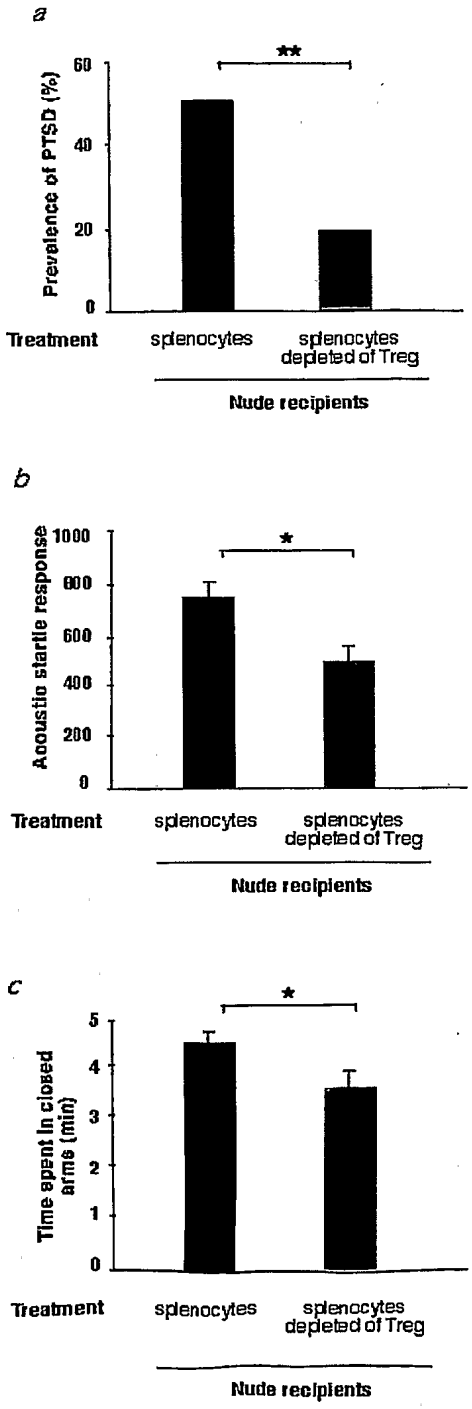

Fig. 7

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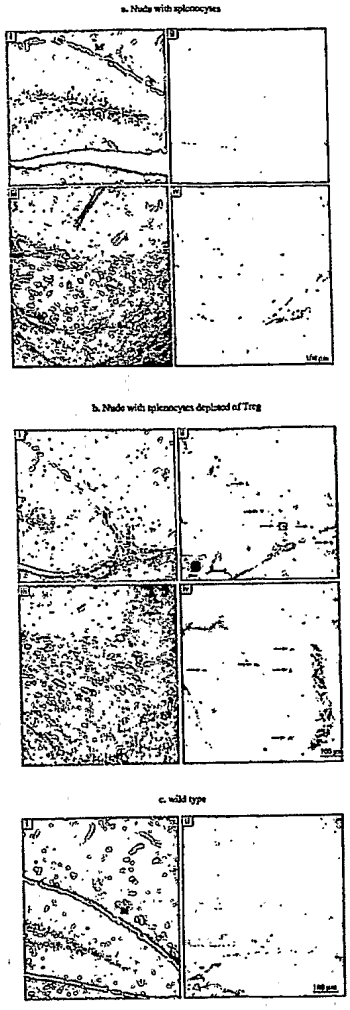

Fig. 8

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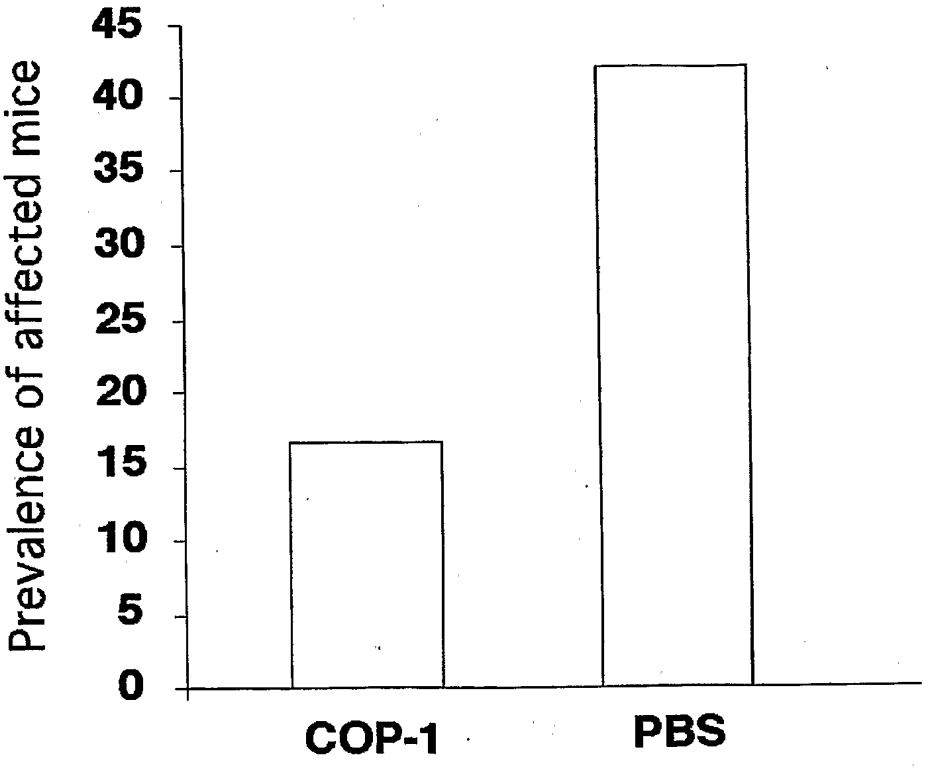

$X^2 = 3.87$, $p < 0.05$

Fig. 9

Copy provided by USPTO from the IFW Image Database on 10/25/2004

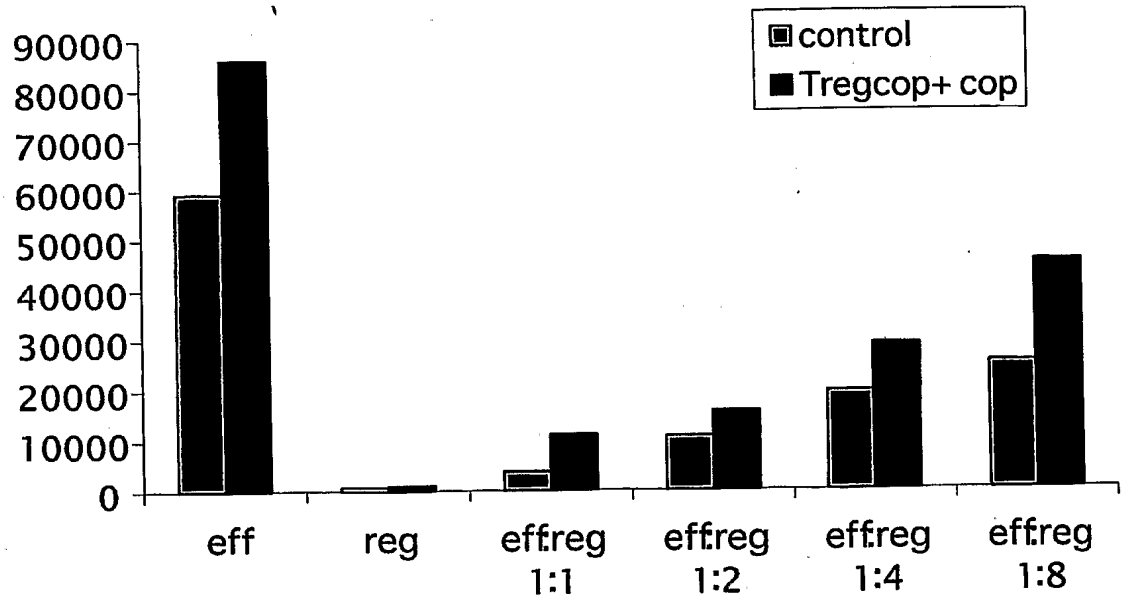

Fig. 10

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